Infectious disease-based pandemic monitoring using distributed sewer water samples

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Introduction

Viral pandemics present a significant threat to public health worldwide, as viruses can be highly infectious and lead to severe disease in high-risk individuals. A significant proportion of patients infected with pandemic influenza and coronaviruses shed viral RNA in their stools. Monitoring of viral RNA levels in wastewater (WW) can help model pandemic progression and decline and have the potential to offer early warning response systems for outbreak detection. The sewer sample surveillance method provides the potential to perform mass testing at a lower cost than conventional, individualized testing. However, there is no standardized testing protocol as yet for monitoring viral RNA in wastewater. Here, we demonstrate a virus concentration and subsequent viral RNA extraction technique from wastewater treatment plant influent samples. The workflow can be further automated on a Beckman Coulter Life Sciences Biomek i5/7 workstation for a robust and reproducible solution for analysis during a viral outbreak.

Methods

Composite 24 hour WW treatment plant influent samples (N=2) were spiked using the enveloped Porcine Reproductive and Respiratory Syndrome (PRRS) virus. Samples were concentrated using either concentration column filtration or polyethylene glycol (PEG) precipitation. RNA was isolated using a column-based viral RNA extraction kit, RNAdvance Viral kit, or TRIzol reagent, following the manufacturer's recommendations. Target virus RNA copy number was quantified by RT-qPCR.

Sample concentration by filtration

40 mL of WW samples with PRRS virus spike-in were centrifugated at 4000 rpm for 10 mins., the supernatants then passed through a 0.45 µM syringe filter to remove larger microorganisms. Samples were concentrated to about 400 µL using a Vivaspin 20 Centrifugal Filter (50 kDa) on centrifugation at 1,500 x g for up to 30 mins. Half of the final concentrated sample was used for the column-based viral RNA extraction kit, and half of the sample was used for the RNAdvance Viral extraction kit.

Sample concentration by PEG precipitation

40 mL of WW samples with PRRS virus spike-in were centrifugated at 4000 rpm for 10 mins. Incubate the supernatant with PEG 8000 at 4 ^{0}C. After overnight incubation, samples were centrifuged at 10,000 x g for 30 mins at 4 ^{0}C. The supernatant was discarded, and PEG pellets were dissolved in 400 µL distilled water. Half of the final concentrated sample was used for TRIzol extraction, and half of the sample was used for the RNAdvance Viral extraction kit.

Workflow

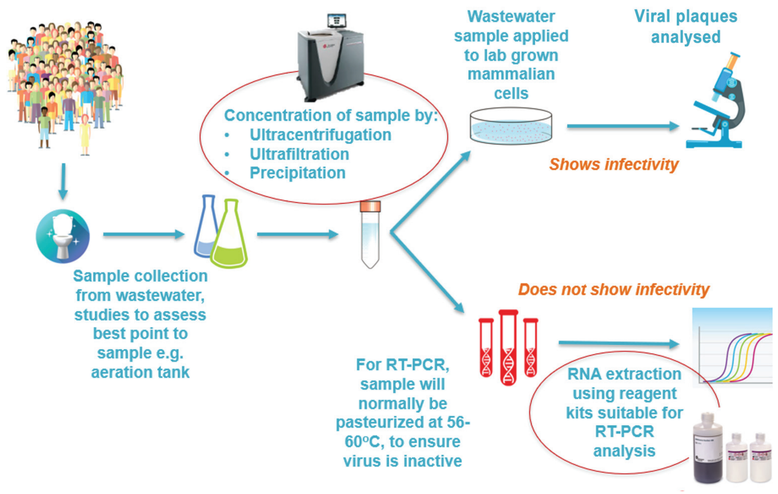

Figure 1. Graphic illustration of the viral wastewater monitoring workflow. First sample is collected and concentrated using either ultracentrifugation, ultrafiltration, or precipitation. RNA then is extracted from the concentrated sample. After viral RNA is extracted, it can be detected in two ways: cell culture methods (e.g., plaque assay) and molecular methods – target detection of RNA with RT-PCR.

Results

We observed comparable Ct values between RNAdvance Viral kit and the column-based viral RNA extraction kit when extracting viral RNA from spiked wastewater samples concentrated using filtration columns. Compared to TRIzol extraction following PEG precipitation, RNAdvance Viral kit showed higher sensitivity and demonstrated consistent results within all test groups.

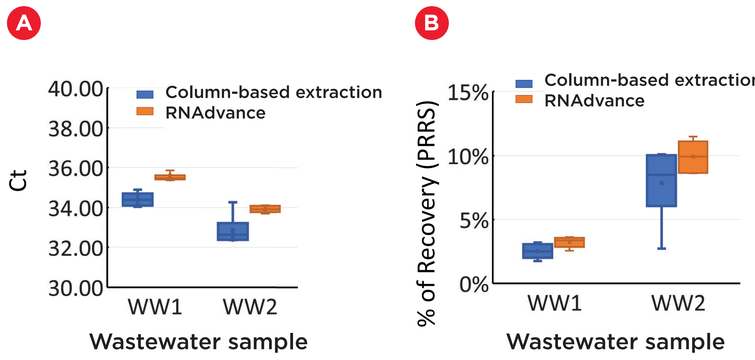

Figure 2. Wastewater samples first concentrated using filtration columns then viral RNA was extracted using either a column-based viral RNA extraction kit or RNAdvance Viral kit. Target virus RNA copy number was quantified by RT-qPCR. (A) Ct value and (B) percentage of PRRS was calculated

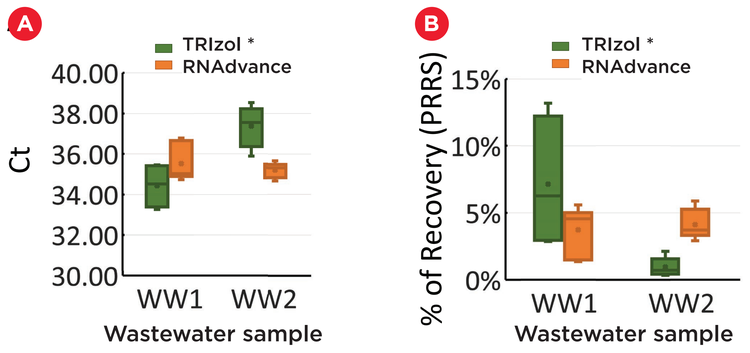

Figure 3. Wastewater samples precipitated using PEG and viral RNA was extracted using either TRIzol reagent or RNAdvance Viral kit. Target virus RNA copy number was quantified by RT-qPCR. (A) Ct value and (B) percentage of PRRS was calculated * 2 out of 6 replicates from TRIzol extraction group showed no amplification and the data points have been omitted from the data set.

Conclusions

In this proof of principle study, we demonstrate an RNAdvance Viral RNA extraction method that is compatible with different wastewater concentration methods. Compared to either the column-based viral RNA extraction kit or TRIzol reagent, RNAdvance Viral RNA extraction kit provided comparable or higher recovery rate. The RNAdvance Viral extraction workflow is also amenable to automation that increases reproducibility and reduces variability.

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